

Product Information

Monoclonal Anti-FKHR (FOXO1a)

Clone FKH117

Purified Mouse Immunoglobulin

Product Number **F6928**

Product Description

Monoclonal Anti-FKHR (FOXO1a) (mouse IgG1 isotype) is derived from the FKH117 hybridoma produced by the fusion of mouse myeloma cells and splenocytes from BALB/c mice immunized with a synthetic peptide corresponding to human FKHR (amino acids 636-651), conjugated to KLH. The isotype is determined using Sigma ImmunoType™ Kit (Product Code ISO-1) and by a double diffusion immunoassay using Mouse Monoclonal Antibody Isotyping Reagents (Product Code ISO-2).

Monoclonal Anti-FKHR recognizes human FKHR (approx. 70-75 kDa). The antibody can be used in ELISA, immunoblotting, and immunoprecipitation.

The superfamily of Forkhead transcription factors (FOX) consists of more than 100 members with orthologues expressed in a variety of species ranging from yeast to man.^{1,2} This superfamily is characterized by a common Forkhead (or Winged Helix) domain, a variant of the helix-turn-helix motif.^{2,3} Forkhead family members have key regulatory roles in embryonic development, differentiation, apoptosis, and tumorigenesis.¹⁻⁵ Three Forkhead family members, termed FKHR (FOXO1a), FKHL1 (FOXO3a), and AFX (FOXO4) were first identified at chromosomal breakpoints in human tumors, and consequently linked to tumorigenesis.⁵⁻⁸ Central to unraveling the role of these proteins was the finding that they are similar in sequence to DAF16 from the nematode *C.elegans*. FKHR, FKHL1, and AFX represent the mammalian counterparts.^{9,10} DAF16 transduces insulin-like and longevity signals.^{9,10} Similar to its mammalian orthologues, it has at least three putative sites for phosphorylation by PKB/AKT.¹⁰

Growth factors regulate the activity of FKHR, FKHL1 and AFX via the PKB/PI3K pathway.^{3,11,12} These transcription factors are inhibited through phosphorylation by PKB, the most likely mechanism being regulation of nuclear localization.¹⁴⁻¹⁶ Phosphorylation of Thr³² and Ser²⁵³ in FKHL1 (FOXO3a) by PKB after induction with survival factors,¹³ results in its retention in the cytoplasm and/or its nuclear exclusion, and thus a subsequent inhibition of FKHL1 dependent transcription.

Survival factor withdrawal results in FKHL1 dephosphorylation and translocation to the nucleus. Within the nucleus, the dephosphorylated FKHL1 induces expression of target genes such as Fas ligand, and triggers apoptosis.¹³ FKHR and AFX were later shown to act by a similar mechanism. FKHR contains three residues, Thr²⁴, Ser²⁵⁶ and Ser³¹⁹ that lie within consensus sequences for phosphorylation by PKB. Likewise, induction by insulin-like growth factor (IGF-1) and insulin leads to phosphorylation of one or more of these sites.^{14,15} Two additional consensus sites for PDK1, at Ser³²² and Ser³²⁵, become phosphorylated in IGF-1-stimulated cells.

A cluster of phosphorylated Ser³¹⁹, Ser³²², Ser³²⁵ and Ser³²⁹ appears to accelerate nuclear export by controlling the interaction of FKHR with the Ran-containing protein complex that mediates this process.¹⁶ In addition to its involvement in apoptosis, FKHR is involved in glucose homeostasis, cell cycle regulation, and as a nuclear receptor cofactor. Interestingly, FKHR was shown to play a role in endometrial differentiation via the Protein kinase A (PKA) pathway.¹⁷

Antibodies reacting specifically with FKHR (FOXO1a) may be used in studying the expression and function of the protein, as well as in correlating its expression pattern with physiological functions or pathological conditions.

Reagent

Monoclonal Anti-FKHR (FOXO1a) is supplied as a solution in 0.01 M phosphate buffered saline, pH 7.4, containing 15 mM sodium azide.

Antibody Concentration: Approx. 2 mg/ml.

Precautions and Disclaimer

Due to the sodium azide content, a material safety data sheet (MSDS) for this product has been sent to the attention of the safety officer of your institution. Consult the MSDS for information regarding hazards and safe handling practices.

Storage/Stability

For continuous use, store at 2-8 °C for up to one month. For extended storage, freeze in working aliquots. Repeated freezing and thawing is not recommended. Storage in frost-free freezers is not recommended. If slight turbidity occurs upon prolonged storage, clarify the solution by centrifugation before use. Working dilutions should be discarded if not used within 12 hours.

Product Profile

By immunoblotting, a working antibody concentration of 0.5-1 µg/ml is recommended using total cell extract of COS-7 cells transfected with FKHR expression plasmid.

Note: In order to obtain the best results using various techniques and preparations, we recommend determining the optimal working dilution by titration.

References

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